THERMOINACTIVATION OF VIRUSES
Announcement II: ON THE NATURE OF ABNORMALITIES IN THE DYNAMICS OF VIRAL INACTIVATION

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The mechanism was studied of the origin of dynamic inactivation irregularities of the viruses of the Venezuelan Equine Encephalitis (VEE), Sindbis and vesicular stomatitus (VVS), which are formed at 50-55° in the first weeks and months after reproduction. The heterogeneity of the viral population is based on the described phenomenon. The t^r variant of VEE, isolated from the initial population, was characterized by significant $\Delta H^{\neq}_{pr}$=95.4 kilocalorie/mole, $\Delta S^{\neq}_{pr}$=219.4 E.E. and $\Delta H^{\neq}_{nk}$=26.02 kc/mole, ΔS_{nk}=8.15 E.E. At 52-56°, the t^r variant of VEE was inactivated in contrast to the initial strain by the "nuclein" type that also caused an inactivation dynamics disturbance. The disappearance of the phenomenon after viral incubation at 4° was related to a lowered stability in these conditions of the t^r viral variant and to its gradual disappearance from the viral population.

The dynamics of the inactivation of viruses are graphically presented by one of three basic forms of curves [5], The formation of any form of curves is caused by the properties of the viral particles, by the presence

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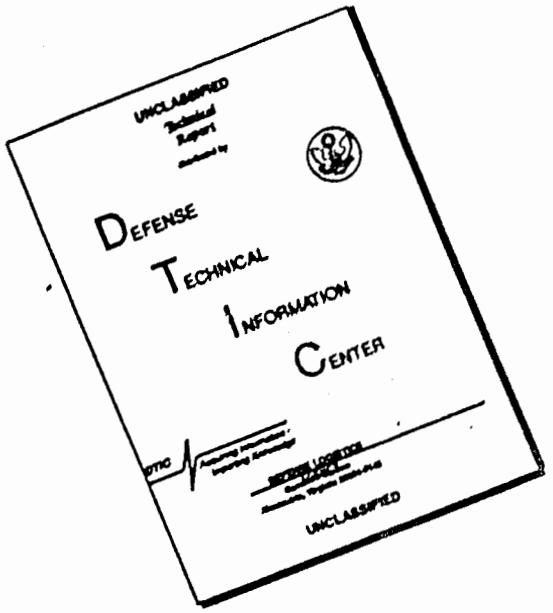

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or absence of a membrane, temperature, during which the inactivation is carried out, the composition of the medium and other factors. The most important moment, which determines the course of the process of the loss of infectious activity of viruses during the influence of the various temperatures, is the relation of the "protein" and the "nuclein" types of inactivation [3].

Relying on the definition of the basic thermodynamic parameters, we tried to discover the mechanism of the origin of dynamic inactivation irregularities of the viral infections, developed in a series of instances at 50-55°. Research was carried out on several RNA-containing viruses, and VEE was used as the basic model.

MATERIAL AND METHODS

We have given a detailed description of the material and methods earlier. The viruses were warmed according to the usual method [2] with a quick titration.

RESULTS

The nature of the irregularity of the dynamics of thermoinactivation of viruses. During the thermal inactivation of some RNA-containing viruses, we obtained irregular graphs of the dependence of the infectious activity of the viruses on the warming period at the same time as the classical curves of inactivation. In fig. 1, a, b, c, examples of such graphs of viral inactivation of VEE, Sindbis and VVS are given. After the influence of temperature for 30-40 min., in the course of which the activity of the viruses fell by 3-5 lg from the initial level, during which this fall went

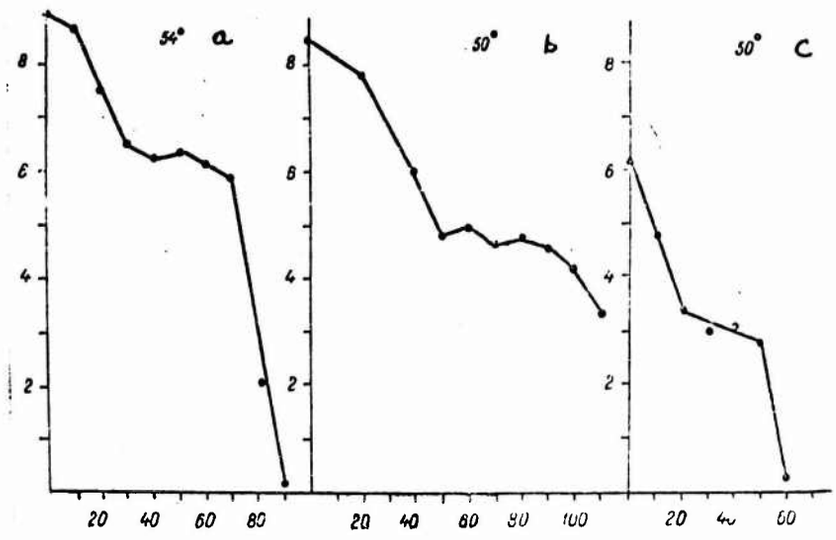

Fig. 1. Dynamics irregularity of thermoinactivation of VEE (a), Sindbis (b) and VVS (c). Here and in figs. 2-4, 6, 7: abcissa--inactivation time (in min.); ordinate--viral activity in lg VIU/ml.

in accordance with the usual dynamics of thermoinactivation, and the expressed plateau. The duration of maintenance of the latter varied from 20 to 30 min., and then it was changed with a period of rather quick inactivation of the viruses.

The described irregularity of the dynamics of inactivation for the Sindbis and WS viruses was observed as correct with a warming temperature of 50-52° and for VEE of 52-55°. Such a feature of inactivation was obtained successfully and regularly in the viruses in the course of the first weeks and months after reproduction. The quickest irregularity disappeared with the warming of the WS. In fig. 2a the change of the dynamics of inactivation of VSV is shown in relation to the period of its being maintained at 4°.

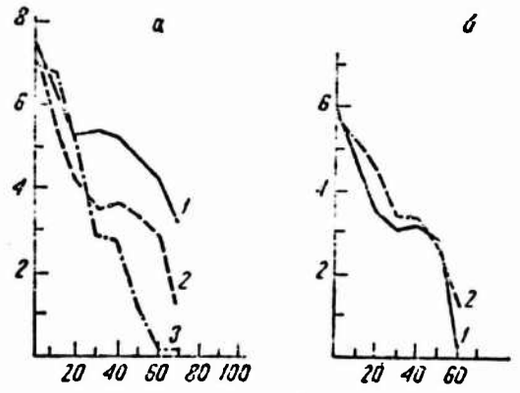

Fig. 2. Dynamics of inactivation of WS in medium No. 199 with 2% albumin serum at 50° used for testing after 1 day (1), after 1 week (2) and after 2 weeks (3) after the reproduction (a). Dynamics of inactivation of WS heated immediately after reproduction at 50° in medium No. 199, cultivated as a physiological solution (1) and a magnesium sulfate solution (2)--b.

The addition of 12.5% magnesium sulfate to the heated virus-containing fluid (fig. 2b) to some degree changed the dynamics of thermoinactivation, and the curve smoothed out somewhat.

Influence of the formation of ribonuclease. With the aim of determining the relationship of the irregular plateau of the viral activity to the ribonuclease, warmed tests processed 20 γ/ml of ribonuclease in 30 min. at room temperature. Experimental and control tests were titrated immediately after the completion of processing. The results of the experiment are shown in fig. 3. The processing of the ribonuclease in the described conditions remove the plateau eventhough the infectious titres were lowered somewhat which reduced the curves of inactivity to an irrelevant smoothness.

Isolation of the thermostable variant of VEE. We assumed that the phenomenon being studied was connected with the heterogeneity of the viral population. Therefore, for a more detailed study of its composition, an attempt was undertaken to isolate the variants of VEE, differing according to symptoms of thermoresistivity (t). For this, the initial virus is held at 54° and, beginning with 40 min, tests are selected with intervals of 10 min. to the space of an hour. The obtained tests were bred in a 100 times

solution of Khenks and passed in the culture of the cells of [illegible] chicken embryos and determined the dynamics of the inactivity of the viral virions at 55°.

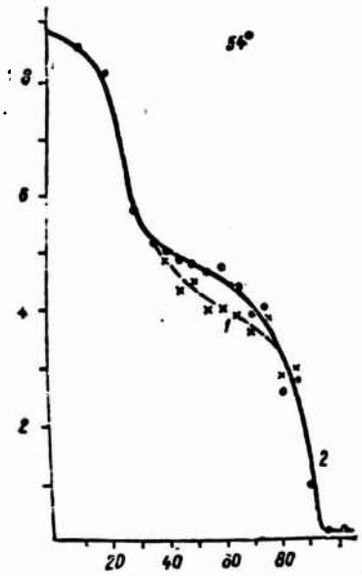

Fig. 3. Influence of processing of RNA on the VEE activity, warmed at 54° for several weeks after reproduction.

1--inactivation after processing with RNA;
2--inactivation without processing with RNA

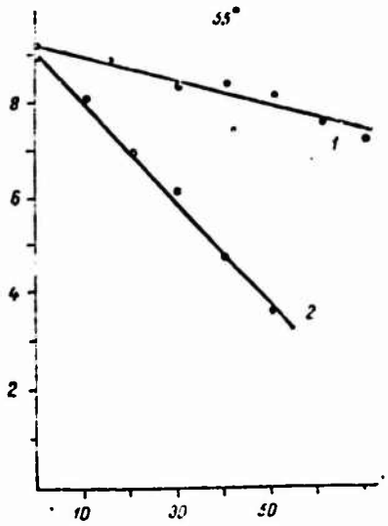

Fig. 4. Dynamics of inactivation at 55° t^r-(1) and t^s-(2) of the variants of VEE.

We were able to obtain the t^r-variant of VEE (strain VEE-80), which is distinctive from the initial t^s-variant significantly by a higher stability to warming at 55°. In fig. 4, the dynamics of the inactivation of the t^s and t^r variants of VEE during warming in medium No. 199 with 2% albumin serum at 55° are shown. When the titres of the initial virus fell to 1 lg for 10 min, the activity of VEE-80 fell to 1 lg for 30-35 min.

Determination of enthalpy and entropy of the activation of the inactivation process of VEE-80. The determination of the dynamics of the inactivation of the infectious activity of VEE-80 during various temperatures from the last calculation of the constant of the speed of inactivation. On the basis of received information, an Arrhenius' dependence was constructed of the logarithim of the constant of the speed of inactivation on the inverse of the absolute temperature (fig. 5), and the significance of the thermodynamical characteristics of the process were determined. Analogous to the

results received for the original t^s-variant of VEE, the dual-component graph proved the existence of various mechanisms of viral inactivation. the "protein" type of inactivation is characterized by significant ΔU=95.4 kcal/mole and ΔS= 219.4 E. E. A smoother, low-temperature portion of the graph ("nuclein type") was described by significant ΔU=26.02 kcal/mole and ΔS=8.15 E.E. Fig. 5 demonstrates a marked removal of the breaking point of high and low temperature components of the graph of Arrhenius' dependence determined for virus VEE-80 in comparison with the original strain. The slope of the steep and smooth parts of the graph are similar for both studied variants of the virus, but their mutual distribution is essentially changed. With a slope greater than 45°, the speed of inactivation of the t^r-variant is lowered even more in comparison with the t^svariant, and at 55-56° VEE-80 is inactivated 5-10 times slower than the t^s-variant. Below 45°, the speed of inactivation of the t^r-variant is greater, and, consequently, in these conditions, the original t^s strain of VEE is more stable.

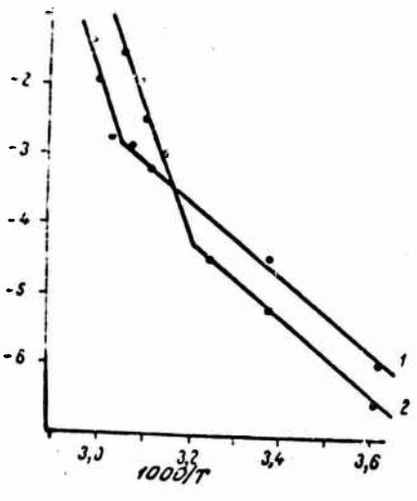

Fig. 5. Arrhenius' dependence of thermoinactivation of the t^r (1) and t^s (2) variants of VEE.
Abcissa--inverse absolute temperature (x 1000);
Ordinate--logarithm of the inactivation constant.

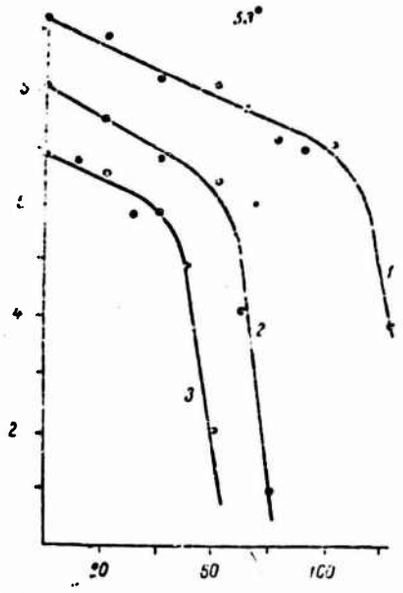

Fig. 6. Inactivation of VEE in medium No. 199 with 2% albumin serum at 53° after 2 (1), 5 (2) and 7 (3) months of maintenance at 4°.

Influence of the duration of maintenance at 4° on the thermoresistance of the VEE. A VEE suspension in medium No. 199 with 2% albumin serum was maintained at 4°. Through the determined intervals of time the virus was warmed to 53°, and the dynamics of the decrease of infectious activity was determined. The results of the experiments, summarized in Fig. 6, show that

maintenance at 4° leads not only to a gradual decrease in the original infectivity of the virus but also changes the dynamics of its inactivation. With an increased period of maintenance, the threshold moment of the period of accelerated inactivation of the virus, related to the process of denaturation of the viral albumins, approach the beginning of the warm-up even more.

DISCUSSION

The dynamics of the inactivation of viruses often becomes the object of discussion among supporters of the "mechanical" and "vitalistic" theories of the mechanics of its formation. The first point of view is based on the relationship to the kinetics of the reactions leading to the loss of infectious activity, the second on the fact of various thermosensitivities of the components of viral populations [5].

On principle, three different forms of the dynamics of viral thermoinactivity are treated. An uninterrupted linear inactivity, corresponding to a reaction of the first order, proves the existence of a single mechanism of infectious loss for viruses inactivated by this type [1]. Two types of dual-component curves of viral thermoinactivity are described. The variant most often met is the one [3,6] by which the initial, accelerated phase of viral inactivation is changed by a period of slow, gradual decrease of infectivity. The most rarely determined is the inverse variant with which the curve has a beginning period of induction and a period following it of accelerated inactivation. VEE [8] is activated according to this type.

The dynamics of inactivation at 50°, described by us above, was characterized by two periods: an initial slow one--"nuclein"; and a later accelerated one--"protein".

The irregular dynamics of the inactivation of several RNA-containing viruses being studied was related, in all probability, to the heterogeneity of the viral population. For VVS, the possibility of the formation of aggregates along 3-12 viral particles was observed which could have dissociated during warming [4]. However, at 50° the disaggregation could have been carried out significantly earlier than the irregular plateau was.

We can not completely deny the role of aggregate reaction in the formation of the researched phenomenon eventhough a special series of tests did not substantiate its existence during the formation of the irregularity. The disappearance of the irregularity after maintaining the viruses at 4° also speaks against the influence of aggregate reaction.

The experiments with the treatment of warming virus-containing suspensions of ribonuclease eliminates the possibility of the formation of a plateau on the curve of the inactivation of VEE with the result of a warm undressing of the RNA virus [6]. At the same time, the comparison of the thermodynamic characteristics of the two distinguished according to thermoresistant

variants of VEE allow us to explain rather satisfactorily the properties of the phenomenon obtained.

Actually, as it follows from the determination of enthalpy and entropy of the activation for VEE-80, the resistance of its albumins to a warm denaturation is higher and noticeably reduced to the action of a lower temperature. The irregularity of the dynamics of thermoinactivation is formed only in the limits of temperatures when it is possible to notice a marked separation of the speed of inactivation forming the viral population of the viral variants differing according to temperature. For example, for VEE both variants lower than 50-52° are inactivated by the "nuclein" type and higher than 56-57° by "protein." Within the limits of 52-56° t^r variant t^s is inactivated as a result of albumin denaturation since variant t^r even still continues to lose its infectivity by the "nuclein" type.

Some smoothing of the curve with the addition of 12.5% magnesium sulphate, arising because of the stabilization of the main stages of inactivation, supports the suggested proposition if it takes into account that the magnesium sulphate stabilizes just the "protein" type of viral inactivation.

Comparison of the constants of speed of inactivation of the t^r and t^s variants of VEE with the temperature of maintenance (4°) shows that in those conditions a progressive liberation of the population from the thermostable variant comes about. This besides the acceleration in the process of shortening of the induction period (fig. 6) leads finally to the disappearance of the phenomenon of the irregularity of the dynamics of thermoinactivation, proceeding more or less quickly relation to the nature of the viruses and composition of the viral population.

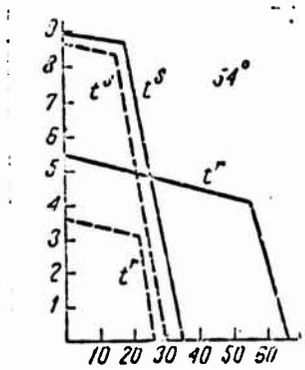

Fig. 7. Diagram of the formation and disappearance of the irregularities of the dynamics of VEE thermoinactivation. Inactivation of viral populations before t^r and after t^s is held at 4°.

Figure 7 illustrates the mechanism of formation of the irregularity of the dynamics of inactivation. Each of the variants of the virus, comprising the population, is inactivated according to the characteristic for VEE at

the given temperature of warming the dual-phase type, described in detail in a previous announcement. During storage at 4°, the [illegible] of inactivation of each variant changes independently of the principle presented in Fig. 5, for which the t^r strain dies out almost 10 times quicker. As a result, the curve of inactivation of the t^r variant, formed in the beginning period after regeneration of the virus, is the irregular plateau during warming since it coincides with the curve of inactivation of variant t^s.

A decreased thermostability during storage of several other viruses is noted [3, 9]. The determination of the Arrhenius dependence for the strains of the foot-and-mouth disease virus was also an inverse dependence between the "protein" and "nuclein types of inactivation [3]. This is of some practical significance during the determination of the time of life of the viruses of the preparations during storage and indicates the possibility of a lowered resistance of the material obtained from the thermostable strains. On the other hand, the possibility of a spontaneous relationship in the viral populations of the t^r and t^s variants of the virus requires a more strict matching of conditions of thermoinactivation which must be oriented for the period of life of the thermoresistant strains.

Comparative investigations of biological properties of the two (t^s and t^r) variants of VEE were developed by us, the results of which will be expressed specially. It is also appropriate here to hold the possible mechanisms of increased thermoresistivity. Besides the existence of an inverse dependence between the levels of resistivity to inactivation according to "nuclein" and "protein" type, the change of the significance of ΔH_{pr} and ΔS_{pr} for variant t^r in comparison to variant t^s draws attention. Probably, for the denaturation of the albumin molecules of the initial viral strain, a gap of less quantity of hydrogen bonds is required than for VEE-80 [10]. Taking into account the role in the process of warm denaturation of the albumins of water molecules capable of forming hydrogen bonds [1], it is possible to introduce a higher thermoresistant VEE as the result of an increase of the limits of stabilization of the particles of its albumin molecules to an inelastic extension under the action of coulomb repulsion and warm oscillation as a result of the formation of additonal inter-molecular hydrogen bonds.

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